under the influence of intestinal bacteria. On the other hand, Mamoli and associates [*Ber.*, **71**, 156, 650, 2083, 2698 (1938)] have demonstrated, in a series of experiments in which the relationship of the starting material to the product of bio-transformation is not subject to question, that bacterial reduction of Δ^4-3-ketosteroids of the hormone series follows an entirely different course, in which the Δ^4-double bond invariably is saturated prior to reduction of the C_3-carbonyl group. The bio-reduction of androstenedione and of testosterone was studied in a number of instances, but in no case was dehydroisoandrosterone or other Δ^5-unsaturated steroid encountered as a reduction product.

Of still greater significance to the question of the origin of the dehydroisoandrosterone found in urine, is the direct experiment of N. H. Callow [*Biochem. J.*, **33**, 559 (1939)]. Callow found that administration of testosterone propionate to a male patient resulted in an unmistakable increase in the urinary excretion of androsterone and 3α-hydroxyaetiocholanone-17, but that there was no evidence of the conversion of the administered Δ^4-3-ketosteroid into dehydroisoandrosterone. Thus the present evidence, in our opinion, is contradictory to Marker's hypothesis.

CONVERSE MEMORIAL LABORATORY
HARVARD UNIVERSITY LOUIS F. FIESER
CAMBRIDGE, MASSACHUSETTS JOHN K. WOLFE

RECEIVED MARCH 26, 1941

A DETERMINATION OF THE HYDROXY AMINO ACIDS OF INSULIN

Sir:

We know of no previous attempts to identify or estimate the hydroxy amino acids of insulin. This is not surprising, since the material is not cheap, and suitable methods have been lacking.

On the basis of our observation[1] that periodic acid reacts, under suitable conditions, rapidly and quantitatively with hydroxy amino acids in the manner shown

$$\mathrm{RCH(OH)-CH(NH_2)-CO_2H} \xrightarrow{HIO_4} \mathrm{RCHO + NH_3 + OCHCO_2H}$$

it has been possible to develop suitable analytical methods. The ammonia evolved may be made to estimate the total of hydroxy amino acids (of the usual types) to be expected. And determinations of the individual aldehydes allow a somewhat accurate appraisal of serine and threonine.[2]

An application of these methods to insulin has given the results shown. All figures given are corrected for moisture and for ash.

TABLE I

"BALANCE SHEET" FOR HYDROXY AMINO ACIDS OF INSULIN[a]

Total hydroxy amino acids		7.75% SE[b,c]
Threonine	2.66%[d]	2.35% SE
Serine	3.57%[e]	3.57% SE
"Other" hydroxy amino acids (as serine)		1.83% SE

[a] Average values. [b] Calculated as "serine equivalent," SE. [c] Actual values, 7.88, 7.62%, SE. [d] Actual values, 2.69, 2.63, 2.68, 2.61, 2.67%. [e] Actual values, 3.52, 3.62%.

Du Vigneaud's excellent summary[3] of the known components of insulin as of 1938, with calculation of "residue numbers" of the amino acids in terms of the Bergmann–Niemann theory, showed 54 units in 288 not accounted for. Calculated in these terms, our results show: threonine, 8 units (found, 7.83); serine, 12 units (found, 11.94); *other* hydroxy amino acids, 6 units (found, 6.12).

It is only proper to add that, since the serine was really determined as formaldehyde, *any part* of the amount reported could be (in equimolecular proportion) hydroxylysine. We think it the simpler assumption that it is all serine.

We wish to express our thanks to Prof. V. du Vigneaud, who gave us the gram of crystalline insulin with which this work was done, and who supplied the data on moisture and ash for this sample which we have used in our corrections.

(1) Nicolet and Shinn, THIS JOURNAL, **61**, 1615 (1939).

(2) Shinn and Nicolet, *J. Biol. Chem.*, **138**, 91 (1941).

(3) Du Vigneaud, *Cold Spring Harbor Symposia on Quant. Biol.*, **VI**, 275 (1938).

BUREAU OF DAIRY INDUSTRY
U. S. DEPT. OF AGRICULTURE BEN H. NICOLET
WASHINGTON, D. C. LEO A. SHINN

RECEIVED APRIL 15, 1941

THE TRIPLE POINT PRESSURE OF HYDROGEN

Sir:

In view of the importance of the hydrogen triple point as a fixed point in thermometry, there is surprising lack of agreement on the value for the triple point pressure. The first five entries of Table I give some determinations of this constant.

The program of the State College Cryogenic Laboratory has involved frequent checks of the laboratory temperature scale against thermometric fixed points. The latter entries of Table I